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The association of TNF-a 308G/A polymorphism and preeclampsia: a meta-analysis

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Abstract

Background: TNF-α 308G/A polymorphism has been the subject of numerous studies, investigating its possible association to preeclampsia with conflicting results. Previous meta-analyses have found no association.

Aim: To update the previous meta-analyses and provide a more comprehensive and reliable conclusion, by performing an exploration of all the possible genetic models of association and subgroup analyses based on disease severity and ethnicity/racial descent.

Methods: A systematic literature search in MEDLINE and EMBASE databases was conducted through July, 2015. Fixed and random effects meta-analysis of the relevant genetic association studies was performed.

Results: 17 eligible studies including 2042 preeclamptic patients and 3612 controls were identified. Overall analysis did not indicate a significant association between TNF-α 308G/A polymorphism and risk for preeclampsia, severe preeclampsia or eclampsia. However, subgroup analysis revealed an elevated risk for preeclampsia among Caucasians, who carry the A allele (FE OR: 1,47, 95% CI: 1,23 to 1,77 under the dominant model).

Conclusion: The role of TNF-α 308G/A polymorphism in preeclampsia susceptibility needs to be reevaluated, as it may account for increased risk for preeclampsia in Caucasians. Future research should determine high risk populations, taking into account genetic, environmental and socioeconomic effects.

Introduction

Preeclampsia (PE) affects 5–8% of pregnancies worldwide, being one of the leading causes of maternal and fetal morbidity and mortality, even in developed countries [1]. Although PE has been the object of intensive research efforts, its precise etiology and pathogenesis remain unknown.

The development of PE is influenced by both genetic and environmental risk factors, suggesting its multifactorial etiology [2]. However, emerging evidence increasingly supports an immunological basis in the pathogenesis of PE [3, 4]. PE is associated with placental disorder, endothelial cell dysfunction and systemic vasospasm. It seems that abnormal immune system activation interferes in the events leading to the former alterations [5, 6]. Adequate placentation requires remodeling of the spiral arteries in the decidua to allow adequate blood flow to the placenta [7, 8]. Placental invasion and development are dependent on tightly regulated local inflammatory processes [9, 10]. In particular, the interaction between fetal trophoblasts and maternal decidual cells is mediated by maternal natural killer (NK) and T-cell activity [5, 11]. Hence, inflammatory cytokines mediate normal placentation in a controlled inflammatory process typical of healthy pregnancy [12]. With PE, there is poor to absent vascular remodeling in the myometrium and decidua. Inadequate placentation results in hypoxia and ischemic–reperfusion injuries, which may lead to the release of inflammatory cytokines [8]. Excessive placental stimuli cause the exaggerated

systemic inflammatory response coupled with generalized endothelial dysfunction, which characterizes PE [3, 12]. Both the innate and adaptive arms of the immune system are shown to be involved, as altered plasma levels of inflammatory cytokines, chemokines and adhesion molecules are found in preeclamptic women [13], both preceding [10, 14] and at the time of diagnosis [9].

Tumor necrosis factor (TNF)-α is a potent and multi-functional cytokine produced by macrophages, lymphocytes and by trophoblast cells during pregnancy. It is responsible for a wide range of proinflammatory actions and its involvement in various autoimmune and inflammatory diseases is well documented. Furthermore, it plays an important role in insulin resistance and other components of the metabolic syndrome, such as obesity, dyslipidemia and endothelial activation [15], which are also common features of PE. Numerous studies have found significantly elevated TNF-α concentrations in plasma and amniotic fluid of preeclamptic patients [16-27]. In addition, TNF-α protein concentrations and m-RNA expression were found to be higher in preeclamptic than normal placentas [28]. Other studies have shown the powerful local (autocrine and paracrine) actions, exerted by TNF-α at the maternal–fetal interface. Macrophages, residing in excess in the placental bed of preeclamptic women, are able to limit extravillous trophoblast invasion of spiral arterial segments through apoptosis, mediated by the combination of TNF-α secretion and tryptophan depletion [29]. In first trimester villous explant cultures TNF-α inhibits trophoblast invasion by induction of plasminogen activator inhibitor-1 (PAI-1) [30]. Finally, trophoblast cell integrity may be compromised, resulting in PE, by stimulation of fibrin production mediated by TNF-α via up-regulation of prothrombinases [31].

The gene encoding TNF-α lies within the class III region of the major histo-compatibility complex (MHC) on the short arm of chromosome 6 (6p21.3). TNF-α 308G/A polymorphism lies in the promoter region of the TNF-α gene and was found to be functionally important by a number of studies. *In vitro* the mutant allele A has been associated with higher TNF-a production [32], in accordance with studies reporting increased transcriptional activity compared to the common (wild type) allele G [33-34]. Furthermore, the –308A TNF-α variant has been associated clinically with higher protein levels in various diseases [36-39]. However, other studies did not confirm a significant difference in the transcriptional activity of mutant allele A and wild type allele G [40, 41].

308G/A was considered to be a variation of great biological significance within the TNF-α gene. The underlying hypothesis is that the TNF-α 308G/A polymorphism can influence the risk for PE through increased production of TNF-α at the maternal–fetal interface in mutant allele carriers. Hence, this SNP has been the subject of numerous studies, which investigated its possible association to PE with conflicting results. However, meta-analysis seems to be conclusive; no association was found between TNF-α 308G/A polymorphism and risk of PE [42-44].

We proceeded in performing a new meta-analysis on TNF-α 308G/A polymorphism and risk of PE for the following reasons: first, there is accumulation of evidence coming from more recent gene association studies, which should be evaluated in the context of on-going research; second, previous meta-analyses did a limited exploration of the possible genetic models of association between TNF-α 308G/A polymorphism and PE, focusing on dominant model only or allele contrast; third, subgroup analysis based on disease severity was scarce in the relevant bibliography; fourth, subgroup analysis based on ethnicity/racial descent was completely lacking.

Methods

Selection of Studies

All of the studies published before July 2015 were identified by computer based searches of MEDLINE and EMBASE data-bases. The following search criterion was used: ("tumor necrosis factor" or "TNF" or "cytokine" or "inflammation") and ("polymorphism" or "single-nucleotide polymorphism" or "variant" or "mutation" or "genotype" or "haplotype" or "gene association study") and ("preeclampsia" or "gestational hypertension" or "pregnancy hypertension" or "HELLP" or "eclampsia"). There was no language limitation. Any clearly irrelevant studies, case reports, editorials and review articles were excluded. Duplicate publications were considered only once. The remaining articles were carefully read in their entity to determine whether they contained information on the topic of interest. All of the references cited in the studies were also searched manually to identify additional published work not indexed by the MEDLINE and EMBASE databases.

To be included in the analysis, studies had to meet the following criteria: (1) examination of the association between 308G/A TNF-α polymorphism and PE risk; (2) inclusion of both PE cases and non-PE controls; (3) patients with clinically confirmed PE; (4) healthy normotensive pregnant women or multiparous non-pregnant women with no history of PE as controls; (5) inclusion of adequate genotypic data for effect size calculation under all the genetic contrasts. Accordingly, the following exclusion criteria were also used: (1) no healthy control group; (2) non-conformity with the criteria for PE; (3) control group, not abiding to the aforementioned characteristics; (4) full genotype distribution in cases and controls unavailable; (5) duplication of previous publications. PE is currently defined as elevated blood pressure (≥140/90 mmHg on two different occasions at least 6 hours apart) with proteinuria ≥300mg/24h or '+' by dipstick after the 20^{th} week of pregnancy [45]. However, other definitions have been used in the past. As long as all cases in a study presented with clinically confirmed PE by any definition, which encompasses persistent hypertension with quantifiable proteinuria after the 20^{th} week of pregnancy, the study was not excluded. On the contrary, if the control group in a study came from the general population or included males, the study was excluded. All authors with otherwise eligible studies except for full genotype distribution data, were contacted and asked to send the relevant data. Studies, whose authors could not be reached, or failed to provide the data after two consecutive requests, were finally excluded. When an individual author published several articles obtained from the same patient population, only the newest or most complete article was included in the analysis. Non conformity of genotype distribution in healthy controls to Hardy- Weinberg equilibrium (HWE) was not used as an exclusion criterion.

Data Extraction

From each eligible study the following information were extracted using a standardized form: first author, journal, year of publication, ethnicity of the study population, demographics, diagnostic criteria and clinical characteristics, matching or adjustments made for known covariates, genotype method and the number of cases and controls for each 308G/A TNF-α genotype. The frequencies of the alleles and the genotypic distributions were extracted or calculated for both the cases and the controls. In addition, it was recorded whether the genotyping in each study was blinded to clinical status. An attempt was made to contact authors if data presentation was incomplete or if it was necessary to resolve an

apparent conflict or inconsistency in the article. After that, any remaining cases of conflicting evaluations or disagreements on inconsistent data from the eligible studies were resolved through careful reexamination of the full text by the author.

Meta-Analysis

Allele frequencies at TNF-α 308G/A polymorphism from each study were calculated by the allele counting method. HWE was assessed in control groups of each study using the goodness-of fit test (chi-square test), which was considered significant for p<0,05 [46].

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association between the TNF-α 308G/A polymorphism and the risk of PE in allele contrast (A vs G) and dominant, co-dominant, recessive and additive genetic models. Based on the individual ORs, a pooled OR was estimated using fixed effects (FEs, Mantel-Haenszel) and random effects (REs, DerSimonian and Laird) models [47]. Heterogeneity was measured using Cochran's Q test and I^2 statistic, which reflects the percentage of total variation across studies that is due to heterogeneity rather than due to chance [48]. The corresponding p-value of the Q statistic below 0,05 or I^2 > 50% was considered significant heterogeneity. RE modelling assumes a genuine diversity in the results of various studies and incorporates to the calculations a between-study variance. Therefore, when there is significant heterogeneity between studies, the pooled OR deriving from the RE model is more valid.

The publication bias of studies for all the genetic contrasts under investigation was assessed using Egger's regression tests for funnel plot asymmetry [49]. Given that this test is underpowered, it was considered statistically significant for p<0,10 rather than for p<0,05.

The main or overall analysis included all the available data on PE, severe preeclampsia (SPE) and eclampsia (E). Based on disease severity additional subgroup analysis was conducted. Studies addressing only SPE were analyzed as a subgroup. Data on eclamptic patients were also analyzed separately and finally another analysis incorporated data on both SPE and E (SPE/E). Subgroup analysis by ethnicity/racial descent for Caucasians and African Americans was also performed. For the subgroup analysis by ethnicity/racial descent the relevant data were only used when stated clearly by the authors, and not assumed by the geographic position of the study.

A sensitivity analysis was conducted excluding all studies considered of questionable quality. Deviation from HWE of genotypes distribution in controls was the main, but not the sole, reason for marking a study as of questionable quality. An additional sensitivity analysis examined the effect of the largest studies in the meta-analysis results, excluding them one by one.

Statistical analyses were performed by Review Manager 5.1 by the Cochrane Library, Microsoft Excel 2013 (Microsoft, Redmond, Washington, USA) and IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of eligible studies

A total of 170 articles relevant to the searched keywords were initially identified. There was no language limitation, however all the obtained results were in English. Of these articles, 30

were selected as potentially relevant studies after reading the titles and abstracts [50-59, 61-67, 69-81]. Full texts were then reviewed for a more detailed evaluation. Searching the reference lists produced 2 more potentially relevant studies [60, 68]. The main reasons for exclusion were as follows: 4 papers proved to be irrelevant studies [50-53], 4 studies included cases of both PE and gestational hypertension [54-57], 1 paper was a duplicate publication [58], 2 studies did not provide any genotype data [59-60] and finally in 4 studies a full genotype distribution in cases and controls was not retrieved after attempted contact with the authors [61-64]. From the latter 4, in one study the controls came from the general population, male and female [61] and another [62] was partially duplicated in a newest article [76]. 17 studies met the inclusion criteria and were included in this meta-analysis. 2 articles provided data on 2 separate study ethnicities: Caucasians and African Americans. Thus data were obtained from 19 studies. The study selection process is shown in Figure1.

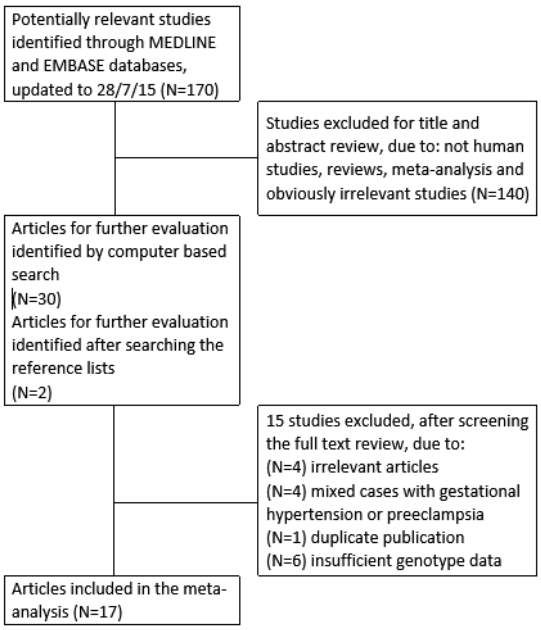


Figure 1*. Flow chart of retrieved studies and studies excluded, with specification of reasons.*

10 articles included cases with PE [65, 68-70, 72, 75, 77-80]. 3 articles were restricted to SPE [66, 73, 81]. 3 articles included distinct cases of PE and E [67, 71, 76]. In the former studies E by definition presupposed PE, so these cases were included in the main analysis. If a study included HELLP syndrome cases indistinctly in the PE group, these were also included in the main analysis. However, only 1 study presented with a distinct HELLP group [74]. It was not stated whether these patients also fulfilled diagnostic criteria for PE. Moreover, the author himself did not include these patients in his meta-analysis on PE. Thus, we have excluded these patients. 10 studies clearly stated the common racial descent of cases and controls: 8 involved Caucasians [67-70a, 73, 74, 79a, 80], 2 involved African Americans [70b, 79b], 1 involved Maya-Mestizo [72], 1 involved Mexican Mullato [76]. The rest of the studies were set in Iran [75, 78], Turkey [71, 77], Brazil [81], UK [65] and USA [66]. The design was case-control in most of the studies. Exceptions were: a prospective cross-sectional study [66], a study nested in a cohort [79] and a study with two arms, longitudinal cohort and cross-sectional [70]. DNA samples used for determination of the TNF-α polymorphism were

TABLE 1. *Characteristics of eligible studies considered in the meta-analysis*.

Ref.	Author, Year	Country	Cases	Controls	Cases / Controls
65.	*Chen, 1996*	UK	Persistent DBP ≥90mmHg with proteinuria ≥300mg/24h or '+' by dipstick	Normotensive pregnant women	14/12
66.	*Livingston, 2001*	USA	Severe PE: SBP≥160 mmHg or DBP ≥110 mmHg on two occasions at least 4 h apart in the presence of proteinuria	Normotensive pregnant women	112/106
67.	*Kaiser, 2004*	Australia (Caucasian)	SBP≥140 mmHg or DBP ≥90 mmHg on two occasions at least 6 h apart and proteinuria ≥300mg/24h or persistent '++' by dipstick (51 cases with E, diagnosed in severe preeclamptic women experiencing convulsions or unconsciousness in perinatal period)	Normotensive primigravidae women	173/100
68.	*Levesque, 2004*	Canada (98% Caucasian)	DBP ≥90mmHg on two occasions at least 4 h apart with proteinuria ≥300mg/24h or persistent '+' by dipstick	Normotensive primigravidae women	175/310
69.	*Saarela, 2005*	Finland (Caucasian)	SBP≥140 mmHg or DBP ≥90 mmHg on two occasions at least 24 h apart with proteinuria ≥300mg/24h	Normotensive primigravidae women	133/115
70a.	*Haggerty, 2005a*	USA (Caucasian)	BP≥140/90 mmHg on two occasions with proteinuria ≥300mg/24h or persistent '+' by dipstick	Normotensive primigravidae women	130/462
70b.	*Haggerty, 2005b*	USA (Afr. American)	BP≥140/90 mmHg on two occasions with proteinuria ≥300mg/24h or persistent '+' by dipstick	Normotensive primigravidae women	20/199
71.	*Pazarbasi, 2007*	Turkey	BP≥140/90 mmHg on two occasions at least 24 h apart with proteinuria ≥300mg/24h or persistent '++' by dipstick (40 cases with E, diagnosed in severe preeclamptic women experiencing convulsions or unconsciousness in perinatal period)	Normotensive primigravidae women	153/80
72.	*Canto- Cetina, 2007*	Mexico (Maya-Mestizo)	BP≥140/90 mmHg on two occasions at least 24 h apart with proteinuria ≥300mg/24h	Normotensive pregnant women	105/200
73.	*Stonek, 2008*	Austria (Caucasian)	SBP≥160 mmHg or DBP ≥110 mmHg on two occasions at least 4 h apart with proteinuria ≥300mg/24h	Normotensive primigravidae women	107/107
74.	*Molvarec, 2008*	Hungary (Caucasian)	Persistent BP≥140/90 mmHg with proteinuria ≥300mg/24h	Normotensive pregnant women	140/144
75.	*Mirahmadian, 2008*	Iran	SBP≥140 mmHg or DBP ≥90 mmHg on two occasions at least 24 h apart with proteinuria ≥300mg/24h or persistent '++' by dipstick	Normotensive pregnant women	160/100
76.	*de Lima, 2009*	Brazil (Mullato)	Persistent BP≥140/90 mmHg with proteinuria ≥300mg/24h (64 cases with eclampsia, defined as otherwise unexplained seizures in preeclamptic women)	Normotensive pregnant women	152/97
77.	*Vural, 2010*	Turkey	SBP≥140 mmHg or DBP ≥90 mmHg on two occasions at least 6 h apart and proteinuria ≥300mg/24h	Healthy non-pregnant women	101/95
78.	*Mohajertehran, 2012*	Iran	Cases with diagnosed PE, no details given	Normotensive pregnant women	54/50
79a.	*Harmon (a), 2014*	USA (Caucasian)	Gestational hypertension (defined as SBP≥140 mmHg or DBP ≥90 mmHg after 2002) and evidence of proteinuria	Normotensive pregnant women	89/827
79b.	*Harmon (b), 2014*	USA (Afr. American)	Gestational hypertension (defined as SBP≥140 mmHg or DBP ≥90 mmHg after 2002) and evidence of proteinuria	Normotensive pregnant women	70/463
80.	*Zubor, 2015*	Slovakia (Caucasian)	SBP≥140 mmHg or DBP ≥90 mmHg on two occasions at least 6 h apart and proteinuria ≥300mg/24h	Normotensive pregnant women	38/38
81.	*Pinheiro, 2015*	Brazil	Severe PE: SBP≥160 mmHg or DBP ≥110 mmHg on two occasions at least 4 h apart with proteinuria ≥2g/24h or at least '++' by dipstick.	Normotensive pregnant women	116/107

extracted from blood. Instead, smears from buccal gingival cells were used in only 1 study [73]. Only 1 study stated that genotyping was blinded to clinical status [73].

The quality of the included studies was assessed on the basis of 5 characteristics: 1) selection criteria for cases; 2) selection criteria for controls; 3) conformity of controls with HWE; 4) level of exclusion of confounding factors; 5) genotyping method. In all but one studies, participants fulfilled generally accepted criteria for PE, SPE and E. Only in 1 study the description of cases was deemed inadequate [78]. Controls were normotensive pregnant women in most of the studies and multiparous non-pregnant women with no history of PE in 1 study [77]. 2 studies, however included two groups of controls: normotensive pregnant women and normotensive non pregnant women [65, 81]. As the obstetrical history of these latter non-pregnant controls was not stated, we chose not to include them in this meta-analysis. The distribution of genotypes in the control group deviated from HWE in 2 studies [66, 75]. However, the corresponding p-value was marginally non-significant in other 3 studies [72, 77, 80]. Some studies included only primigravidae women to match for parity [67, 68, 69, 70, 71, 73]. Matching of cases and controls included gestational age [66, 78], maternal age [77], maternal age, gestational age, BMI, month of year at delivery [68], maternal age, educational level, smoking [73]. 3 studies implemented statistical adjustment to control for maternal age, BMI, primiparity [74], maternal age, smoking, education [70], maternal age, smoking, BMI, parity, socioeconomic status [79], which did not change the pattern of their results. Moreover, these latter 2 studies stratified data by race. In 6 studies there is no description of specific actions taken to exclude confounding factors [65, 72, 75, 76, 80, 81]. Validated genotyping methods were used in all of the studies; namely, PCR- RFLP [66, 67, 69, 71, 72, 74, 77, 78, 80], PCR with allele-specific probes [68, 75, 76, 81], quantitative PCR [65], real-time PCR [70], microarrays [73] and custom GoldenGate Genotype Assay [79]. A sensitivity analysis was performed excluding all studies considered as of low quality according to the aforementioned characteristics [65, 66, 72, 75, 77, 80].

Detailed characteristics of the included studies are summarized in Table 1 and Table 2.

Main Results, Subgroup and Sensitivity Analyses

Overall, the ORs and 95% CIs of PE risk were considered under allele contrast (A vs G) and dominant, co-dominant, recessive and additive genetic models. A summary of all the meta-analysis findings of the associations between TNF-α 308G/A polymorphism and PE risk is provided in Table 3.

A total of 5654 subjects from 19 (17) studies were involved in the main analysis, including 2042 PE cases and 3612 controls. The meta-analysis results showed that TNF-α 308G/A polymorphism was not linked to the risk of PE under any genetic model (RE OR: 1,20, 95% CI: 0,90 to 1,58 under the dominant model). Nonsignificant heterogeneity among studies was found only under the additive and the recessive model (P=0,39; I^2=23% and P=0,71; I^2=0% respectively). Main analysis results are also presented in Figure 2.

490 cases and 597 controls, 1087 subjects in total, from 6 studies, were included for data synthesis regarding the TNF-α 308G/A polymorphism and its relationship to SPE/E. The association was not significant under any genetic model (RE OR: 1,10, 95% CI: 0,65 to 1,87 under the dominant model). Only the additive and recessive models showed nonsignificant heterogeneity among studies (P=0,48; I^2=0% and P=0,71; I^2=0% respectively). 655 subjects

TABLE 2. *The distribution of the 308G/A TNF-α genotype for cases and controls (in parenthesis are the respective percentages).*

	Distribution of 308G/A TNF Genotype						**Allele Frequency (%)**				
	GG		GA		AA		G		A		P HWE
Ref.	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Controls
65.	9 (64)	2 (16,6)	4 (29)	7 (58,3)	1 (7)	3 (25)	78,6	45,8	21,4	54,2	0,54
66.	104 (93)	94 (88,6)	7 (6)	9 (8,4)	1 (0,9)	3 (2,8)	96	92,9	4	7,1	<0,01
67.	196 (72)	67 (67)	70 (25)	29 (29)	7 (2,5)	4 (4)	84,6	81,5	15,4	18,5	0,70
68.	127 (72,5)	236 (77,1)	44 (25,1)	62 (20,2)	4 (2,2)	8 (2,6)	85,1	87,2	14,9	12,8	0,12
69.	97 (73)	94 (81,7)	32 (24)	21 (18,3)	4 (3)	0 (0)	86	90,9	15	9,1	0,28
70a.	79 (61)	338 (73,1)	45 (34,6)	115 (24,9)	6 (4,6)	9 (2)	78	85,6	22	14,4	0,83
70b.	16 (80)	153 (76,9)	4 (20)	44 (22,1)	0 (0)	2 (1)	90	88	10	12	0,55
71.	132 (87)	76 (95)	20 (13)	4 (5)	0 (0)	0 (0)	93,4	97,5	6,6	2,5	0,81
72.	86 (82)	155 (76)	18 (17)	49 (24)	1 (0,95)	0 (0)	90,5	88	9,5	12	0,05
73.	74 (69,1)	77 (72)	31 (29)	26 (24,3)	2 (1,9)	4 (3,7)	83,6	84,1	16,4	15,9	0,34
74.	111 (79,2)	107 (74,3)	24 (17,1)	33 (22,9)	5 (3,5)	4 (2,7)	87,9	85,8	12,1	14,2	0,46
75.	127 (79,3)	100 (100)	33 (20,6)	0 (0)	0 (0)	0 (0)	89,7	100	10,3	0	0,02
76.	123 (80,9)	71 (73,2)	25 (16,5)	24 (24,8)	4 (2,6)	2 (2)	89,1	85,6	10,8	14,4	0,98
77.	81 (80,2)	77 (81)	15 (14,8)	15 (15,8)	5 (4,9)	3 (3,2)	87,6	89	12,4	11	0,05
78.	28 (52)	42 (84)	26 (48)	8 (16)	0 (0)	0 (0)	76	92	24	8	0,53
79a.	51 (57,3)	595 (72)	34 (38,2)	217 (26,2)	4 4,5	15 (1,8)	76,4	85	23,6	15	0,34
79b.	59 (84,3)	347 (75)	11 (15,7)	103 (22,2)	0 (0)	13 (2,8)	92,1	86	7,9	14	0,12
80.	22 (57,9)	30 (79)	15 (39,5)	6 (15,8)	1 (2,6)	2 (5,2)	77,6	86,8	22,4	13,2	0,05
81.	88 (75,8)	77 (72)	26 (22,4)	25 (23,4)	2 (1,7)	5 (4,6)	87	83,6	13	16,4	0,13

in total, 335 cases and 320 controls, from 3 studies, were included for data synthesis on TNF-α 308G/A polymorphism and risk of SPE. Again, no association was found (FE OR: 1,03, 95% CI: 0,69 to 1,54 under the dominant model). However heterogeneity was not significant in all but one the genetic contrasts (P=0,37; I^2=0% under the dominant model). For E, a total of 432 subjects, 155 cases and 277 controls, from 3 studies, were included for data synthesis. The meta-analysis did not produce any significant association (RE OR: 1,49, 95% CI: 0,45 to 4,93 under the dominant model). Nonsignificant heterogeneity among studies was found only under the additive and the recessive model (P=0,40; I^2=0% and P=0,64; I^2=0% respectively). Subgroup analysis results based on disease severity (SPE/E, SPE, E) are also presented in Figure 3.

Table 3. *ORs and heterogeneity results for the genetic contrasts of 308G/A TNF polymorphism for PE (main analysis, sensitivity analysis for poor quality of studies, subgroup analysis based on*

Genetic Contrast/ Model	Population	FE OR (95% CI)	RE OR (95% CI)	Stu-dies	I^2 (%)	P Q Test
A vs G	All	0,99 (0,82 to 1,21)	1,05 (0,75 to 1,47)	19	64,4%	<0.01
Allele frequencies	Sensitivity	0,95 (0,76 to 1,17)	1,01 (0,68 to 1,50)	13	68,7%	<0.01
	Caucasians	1,30 (0,99 to 1,71)	1,30 (0,99 to 1,71)	8	0%	0,57
	African Americans	0,66 (0,34 to 1,25)	0,66 (0,34 to 1,25)	2	0%	0,50
	SPE/E	0,87 (0,78 to 0,97)	0,91 (0,67 to 1,24)	6	85%	<0.01
	SPE	0,83 (0,71 to 0,97)	0,78 (0,56 to 1,09)	3	75%	0,02
	E	0,92 (0,78 to 1,08)	1,13 (0,60 to 2,11)	3	92%	<0.01
Dominant	All	1,22 (1,05 to 1,41)	1,20 (0,90 to 1,58)	19	68%	<0.01
AA, GA vs GG	Sensitivity	1,29 (1,10 to 1,51)	1,27 (0,96 to 1,69)	14	65%	<0.01
	Caucasians	1,47 (1,23 to 1,77)*	1,46 (1,17 to 1,84)*	8	32,6%	0,16
	African Americans	0,61 (0,34 to 1,10)	0,61 (0,34 to 1,10)	2	0%	0,55
	SPE/E	1,09 (0,81 to 1,46)	1,10 (0,65 to 1,87)	6	65%	0,01
	SPE	0,93 (0,64 to 1,36)	0,93 (0,64 to 1,36)	3	0%	0,37
	E	1,39 (0,87 to 2,22)	1,49 (0,45 to 4,93)	3	81%	<0.01
Co-Dominant	All	1,22 (1,05 to 1,42)	1,21 (0,93 to 1,59)	19	62,9%	<0.01
GA vs GG, AA	Sensitivity	1,29 (1,10 to 1,51)	1,27 (0,98 to 1,66)	13	58%	<0.01
	Caucasians	1,43 (1,18 to 1,73)*	1,43 (1,14 to 1,78)*	8	29%	0,19
	African Americans	0,70 (0,39 to 1,26)	0,70 (0,39 to 1,26)	2	0%	0,65
	SPE/E	1,16 (0,85 to 1,58)	1,16 (0,71 to 1,92)	6	57%	0,04
	SPE	1,03 (0,69 to 1,54)	1,03 (0,69 to 1,54)	3	0%	0,61
	E	1,37 (0,85 to 2,21)	1,45 (0,45 to 4,70)	3	79%	<0.01
Additive	All	1,14 (0,76 to 1,70)	1,11 (0,73 to 1,70)	19	23%	0,39
AA vs GG	Sensitivity	1,15 (0,74 to 1,79)	1,08 (0,63 to 1,83)	13	23%	0,23
	Caucasians	1,43 (0,87 to 2,34)	1,39 (0,79 to 2,45)	8	19,5%	0,27
	African Americans	0,66 (0,08 to 5,50)	0,68 (0,06 to 6,95)	2	17,3%	0,27
	SPE, E	0,64 (0,29 to 1,44)	0,68 (0,30 to 1,55)	6	0%	0,48
	SPE	0,39 (0,14 to 1,13)	0,39 (0,14 to 1,13)	3	0%	0,92
	E	1,50 (0,41 to 5,52)	1,58 (0,42 to 5,91)	3	0%	0,40
Recessive	All	1,15 (0,78 to 1,70)	1.15 (0,78 to 1,70)	19	0%	0,71
AA vs GG, GA	Sensitivity	1,19 (0,77 to 1,84)	1,28 (0,82 to 2,00)	13	0%	0,45
	Caucasians	1,42 (0,87 to 2,31)	1,42 (0,87 to 2,31)	8	0%	0,50
	African Americans	0,70 (0,086 to 5,79)	0,72 (0,075 to 6,98)	2	13,3%	0,28
	SPE/E	0,60 (0,27 to 1,34)	0,62 (0,27 to 1,40)	6	0%	0,71
	SPE	0,39 (0,14 to 1,12)	0,39 (0,14 to 1,13)	3	0%	0,94
	E	1,22 (0,34 to 4,41)	1,23 (0,34 to 4,51)	3	0%	0,64

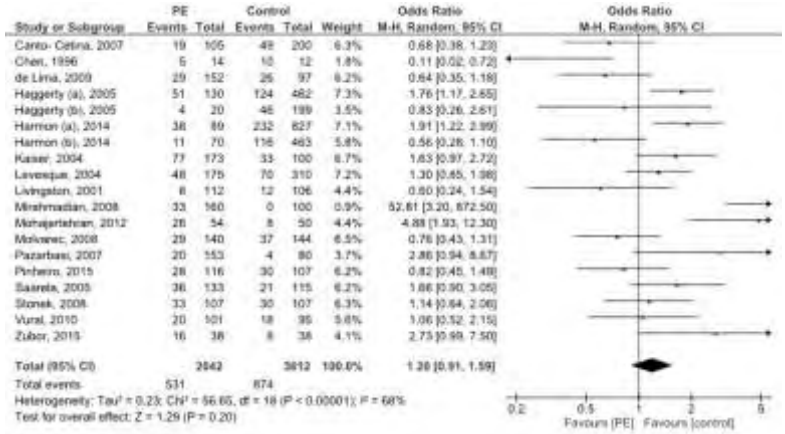
ethnicity/racial descent and disease severity).

Subgroup analysis based on ethnicity/racial descent included 3108 Caucasians, 1005 cases and 2103 controls, from 8 studies, and 752 African Americans, 90 cases and 662 controls, from 2 studies. For African Americans heterogeneity among studies was not significant (P=0,55; I^2=0% under the dominant model) and no association was found (FE OR: 0,61, 95% CI: 0,34 to 1,10 under the dominant model). In Caucasians, the subgroup analysis results showed that the TNF-α 308G/A polymorphism was linked to the risk of PE under the dominant and co-dominant models (FE OR: 1,47, 95% CI: 1,23 to 1,77, dominant model; FE

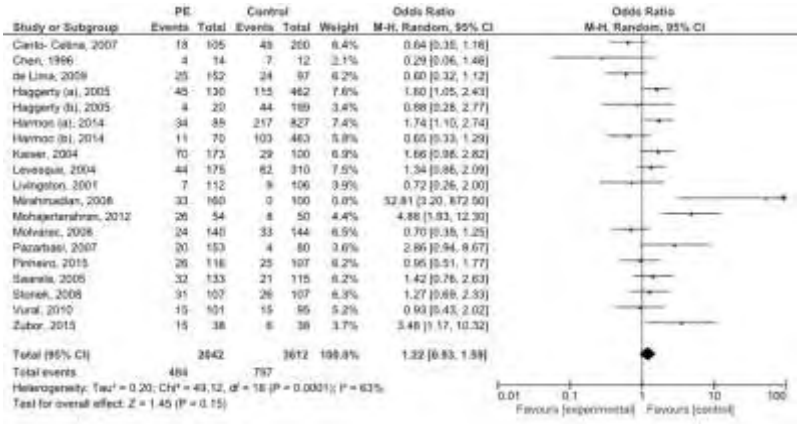
OR: 1,43, 95% CI: 1,18 to 1,73, co-dominant model). It is to be underlined that in this subgroup the heterogeneity among studies was not significant in any case (P=0,16; I^2=32,6%

Figure 2. *RE OR estimates with the corresponding 95% CI for the dominant (A), co-dominant (B), recessive (C) and additive (D) models of TNF 308G/A polymorphism allele A and the risk of PE.*

A



B



C

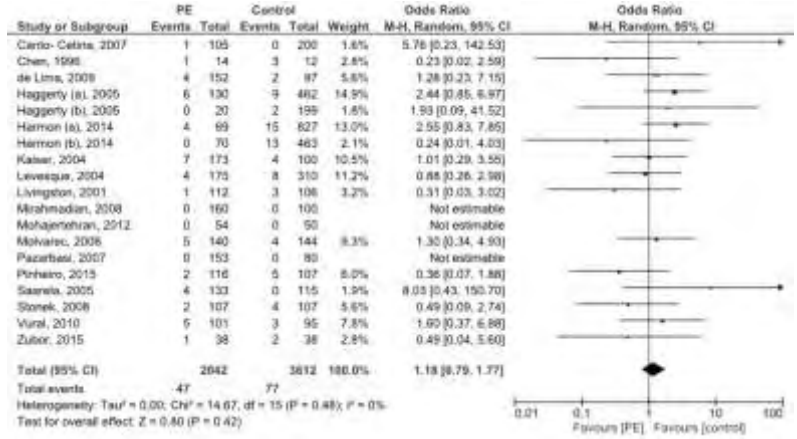
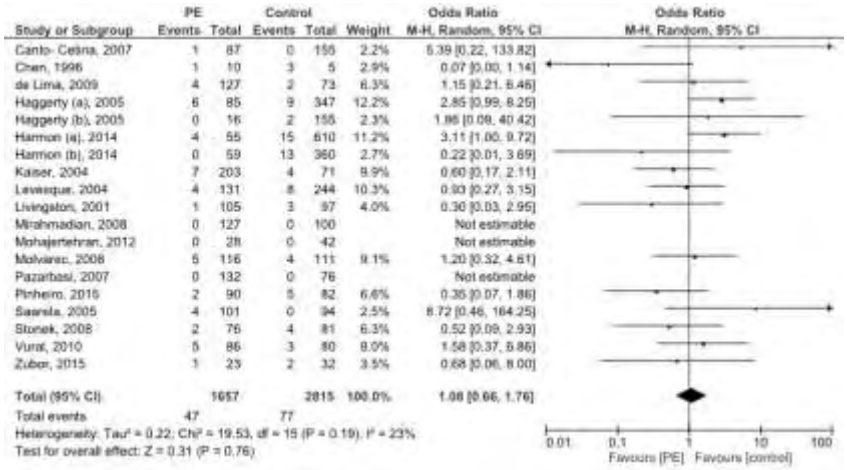


Figure 2. *(continued)*

D



and P=0,19; I^2=29% for dominant and co-dominant model respectively). Subgroup analysis results for Caucasians are also presented in Figure 4.

Sensitivity analysis excluding studies of poor quality and sensitivity analysis excluding one by one the largest studies did not alter the pattern of results in the main analysis and the subgroup analyses under any contrast.

Potential Bias

Begg's funnel plot and Egger's tests were performed for all the genetic models under investigation in order to assess the publication bias of included studies (Figure 5). The shapes of the funnel plots did not reveal any evidence of obvious asymmetry under the co-dominant, recessive and additive model. Egger's test did not show any significantly statistical evidence of publication bias under any contrast (dominant model, p= 0,57; co-dominant model, p= 0,85; recessive model, p= 0,12; additive model, p= 0,17). Furthermore, the possibility for subgroup-specific publication bias was assessed for Caucasians under the dominant model (Egger's test, p=0,91). Thus, it can be assumed that the risk of publication bias in this meta-analysis is low.

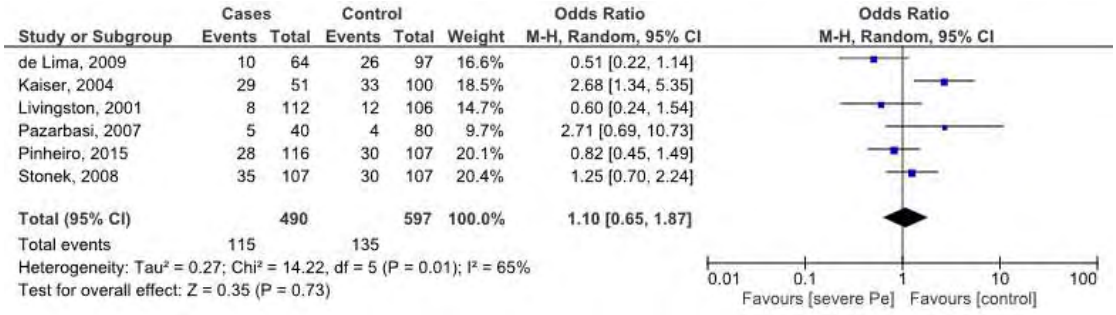
Discussion

This meta-analysis examined the TNF-α 308G/A polymorphism and its relation to susceptibility for PE. The strength of the present analysis was based on the accumulation of published data giving greater information to detect significant differences. In the present study, the effect of allele frequency and the effects of the dominant, co-dominant, additive and recessive models were estimated. Much of this work has been presented for the first time. Disease severity in PE was taken into account, in our attempt to form less heterogeneous groups that would to lead us to more consistent results. In addition, the

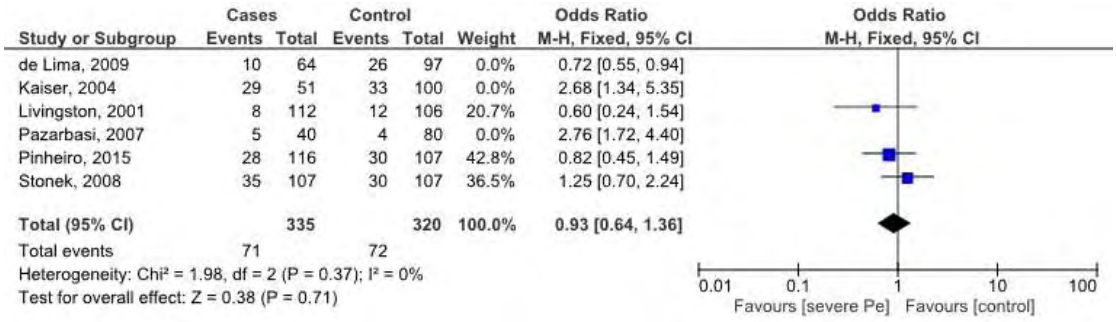
consistency of genetic effects across populations with different ethnicity/racial descent was investigated, also for the first time to our knowledge.

Figure 3. *TNF 308G/A polymorphism and risk for SPE/E (A), SPE (B) and E (C). Analysis based on the dominant model.*

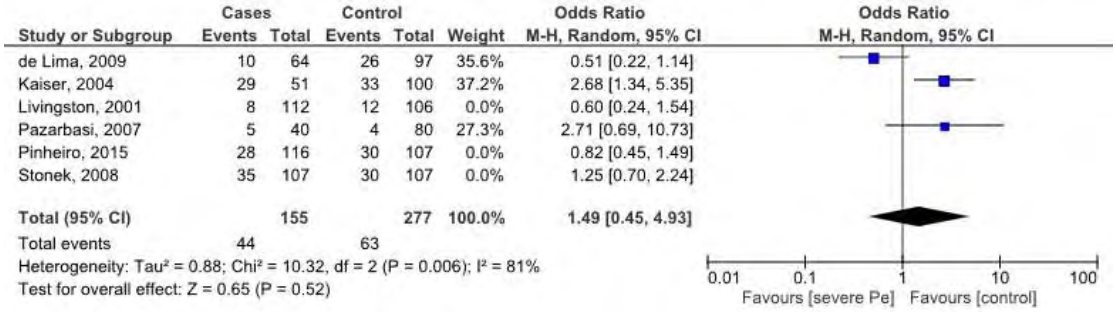
A



B



C

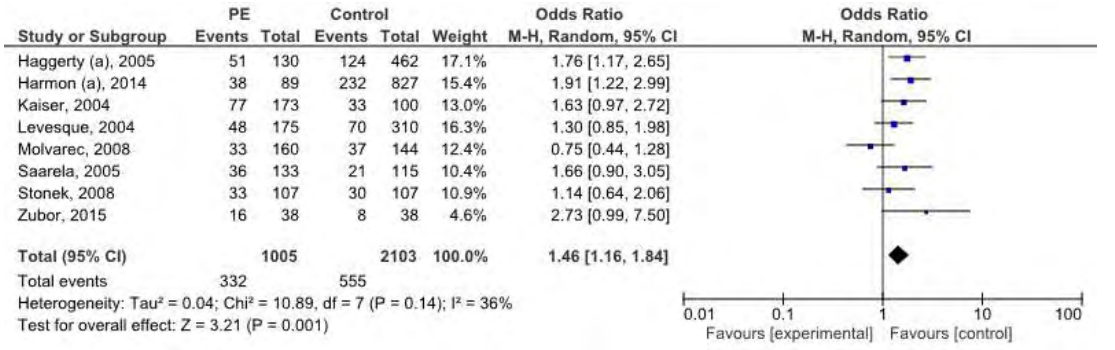


Our overall analysis results are consistent with previous meta-analysis [42-44], which failed to associate TNF-α 308G/A polymorphism with PE risk. SPE and E patients theoretically represent a more homogeneous group. However, TNF-α 308G/A polymorphism does not seem to affect the risk for SPE/E, SPE or E either. This result, too, is in accordance with previous, eventhough less extended, meta-analysis, which found no association between TNF-α 308G/A polymorphism and SPE/E risk [42]. Subgroup analysis based on ethnicity/racial descent, performed for the first time, revealed an association between TNF-

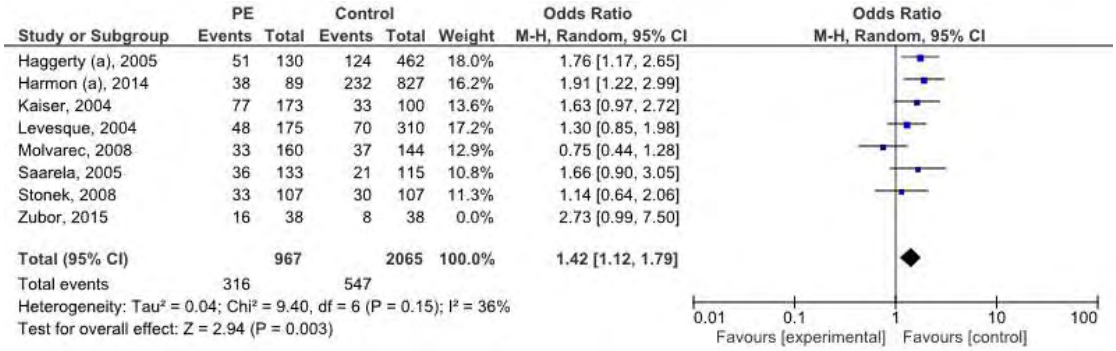
α 308G/A polymorphism and risk of PE in Caucasians. Moreover, within this subgroup heterogeneity among studies was invariably nonsignificant. The result was robust to

Figure 4. *TNF 308G/A polymorphism and risk for PE. Subgroup analysis for Caucasians based on the dominant model (A), with sensitivity analysis for poor quality of studies (B) and sensitivity analysis for large studies (C).*

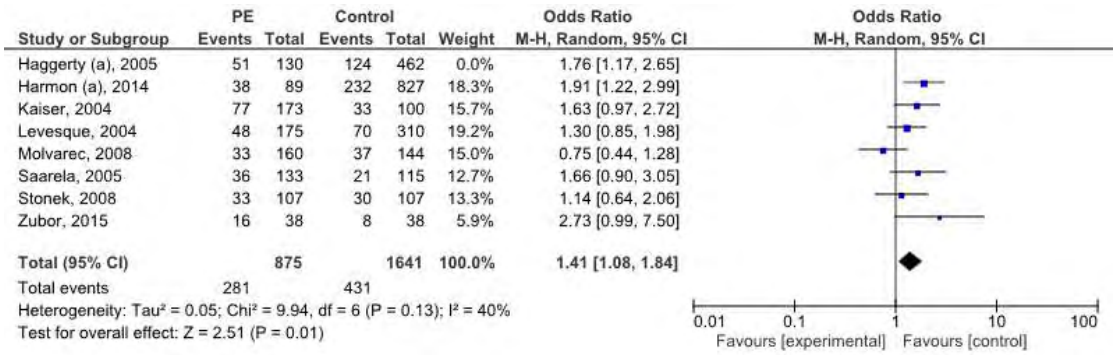
A



B



C



sensitivity analysis. Furthermore, it is unlikely that publication bias is the underlying cause of the positive association found.

Eventhough no individual study has an excessive effect on the subgroup analysis results, the positive association within the subgroup of Caucasians is due in the greatest part to the

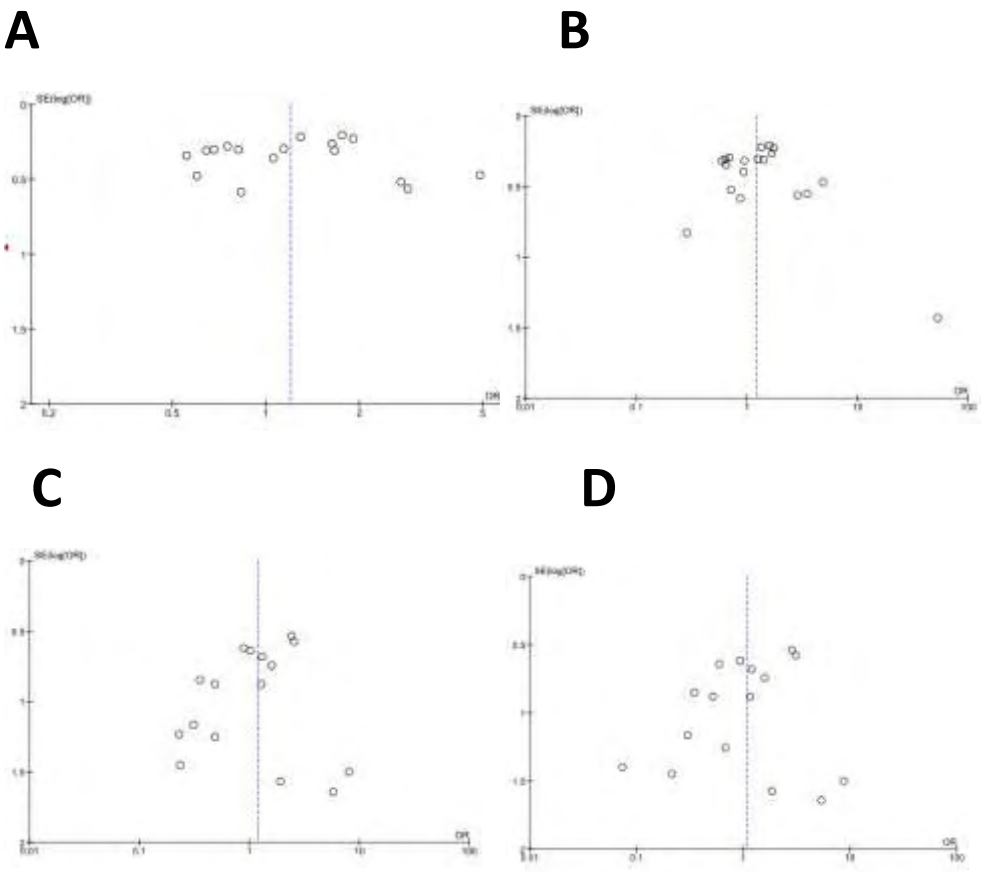
presence of 2 studies [70, 79]. Both of them are set in USA, provide a large sample, stratified by race, and are characterized by a strict methodology. Each of them, individually, found the risk for PE to be increased in Caucasians carriers of the A allele. Neither detected the same association for African Americans. Furthermore, in both studies the results were unaffected by statistical adjustment for known covariates, such as maternal age and smoking. It is unclear why distinctive association patterns emerged for African Americans and Caucasians; it may reflect distinct phenotypes, or possibly interactions with social or environmental exposures that are differentially distributed across these two populations [79]. However, a number of studies can support the hypothesis that known differences in clinical manifestations between various ethnic groups, such as PE prevalence [1], are due in part to inheritance of cytokine polymorphism. Differences of the allelic frequency in TNF-α 308G/A polymorphism and other genes associated with inflammation have been reported among diverse geographically distributed populations [82] and among Whites and African Americans in the United States [83]. Moreover, regulation of TNF-α levels during pregnancy appears to differ between White and African American women [84]. Thus, the claim that patterns of cytokine concentrations, as well as the genetic regulation of these concentrations, may be different between these two latter groups [85] would be in accordance with our results. Concluding, it is high likely that our findings represent a true association and the risk for PE among Caucasians, who carry the A allele in the TNF-α 308G/A polymorphism is indeed elevated.

Although there is a strong genetic contribution to PE, other complex mechanisms and environmental interactions play a significant role in its pathogenesis. Maternal and fetal genes interacting with each other (and a variety of environmental stimuli) interfere on PE severity and outcome. Interactions between genetic susceptibility and environmental exposures such as smoking, infections, nutrition, or exposure to environmental toxicants contribute to the heterogeneity of phenotypes seen in PE [70]. Thus, it is difficult to define genetic variants, which consistently and significantly contribute to PE. Indeed, the effect of various gene polymorphisms on PE, estimated in gene association studies, appears to be small as evidenced by the reported ORs. The repeatability of the results is also poor across different ethnic groups. Therefore, gene polymorphisms explored so far through on-going research have not gained a role in the clinical prediction of PE. Considering the important environmental and lifestyle modifiable risk factors for PE, it will be interesting to investigate potential gene–gene and gene-environment interactions in genes associated with PE. Per example, there is considerable evidence that inflammation may be part of the casual pathway through which obesity predisposes to preeclampsia [86]. Elevated body fat may trigger excessive cytokine production among genetically susceptible pregnant women [87].

It is well documented that women who have had pre-eclampsia are more likely to develop cardiovascular disease, and pre-eclampsia and cardiovascular disease share various risk factors, including obesity, hypertension and diabetes [1]. The shared risk factors for both pre-eclampsia and later-life vascular diseases, as well as the familial segregation of all these disorders suggest that these diseases share a common genetic predisposition that interacts with the environment and may predispose individuals to vascular disorders which manifest at different time points throughout the life course. Prospective cohort studies collecting data on relevant clinical, environmental and lifestyle risk factors coupled with longitudinal measurement of angiogenic, inflammatory factors and genetic variations in candidate genes will be able to determine high risk populations and provide information useful in the clinical prediction of PE.

Our meta-analysis has several limitations, which should be taken into account. First, eventhough our main analysis is not underpowered, lack of sufficient statistical power for some outcomes may still be encountered in subgroup analysis, due to the relatively small sample size used. Second, meta-regression analysis to further control for known

Figure 5*. Funnel plots of SE against the log OR for the association between 308G/A TNF polymorphism and risk of PE. (A) Dominant model (Egger test: t= 0,58, p= 0,57), (B) Co-dominant model (Egger test: t= 0,19, p= 0,85), (C) Recessive model (Egger test: t= 1,61, p=0,12), (D) Additive model (Egger test: t= 1,40, p= 0,17).*



confounders was not undertaken in our study due to the lack of relevant data. Smoking, maternal weight, maternal age, parity and other clinical covariates were not considered by a great number of studies. Such problems, if inherent in the included studies, remain unsolved after meta-analysis. Third, PE is believed to result from a complex interplay between genetic components, maternal and fetal genes interacting with each other, and environmental factors. It was impossible to incorporate to our analysis the small amount of data regarding the involvement of fetal genes in PE risk, disease severity and outcome. Similarly, although some of the studies reported on haplotypes, we did not include these data to our analysis. None of the studies explored gene-environment interactions. Fourth, since some studies were not considered in our analysis due to methodological reasons, we can't tell whether inclusion of their data would have altered our findings, especially the result of subgroup analysis for Caucasians.

As a conclusion, the present meta-analysis aimed to update the previous meta-analyses, as well as to provide a more comprehensive and reliable conclusion on the association between the TNF-a 308G/A polymorphism and risk of PE. Although TNF-a 308G/A polymorphism was not overall significantly associated with PE, SPE or E, its role in PE susceptibility needs to be reevaluated, as it may account for increased risk for PE in

Caucasians. The complexity of TNF-α regulation and apparent heterogeneity based on ethnicity/racial descent support further investigation into this gene. However, future studies need to focus in determining high risk populations, based on a plurality of characteristics, taking into account genetic, environmental and socioeconomic effects.

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